

A. Construction of pvvNADLd1ubiNS2

1. RT-PCR fragment 1
Bovine ubiquitin

2. PCR fragment 2
NS3 to Nsi I

3. Restrict PCR fragment 3
with PinA I and Nsi I

4. Ligate fragment 3
with pvvNADLd1NS2
restricted with PinA I
and Nsi I

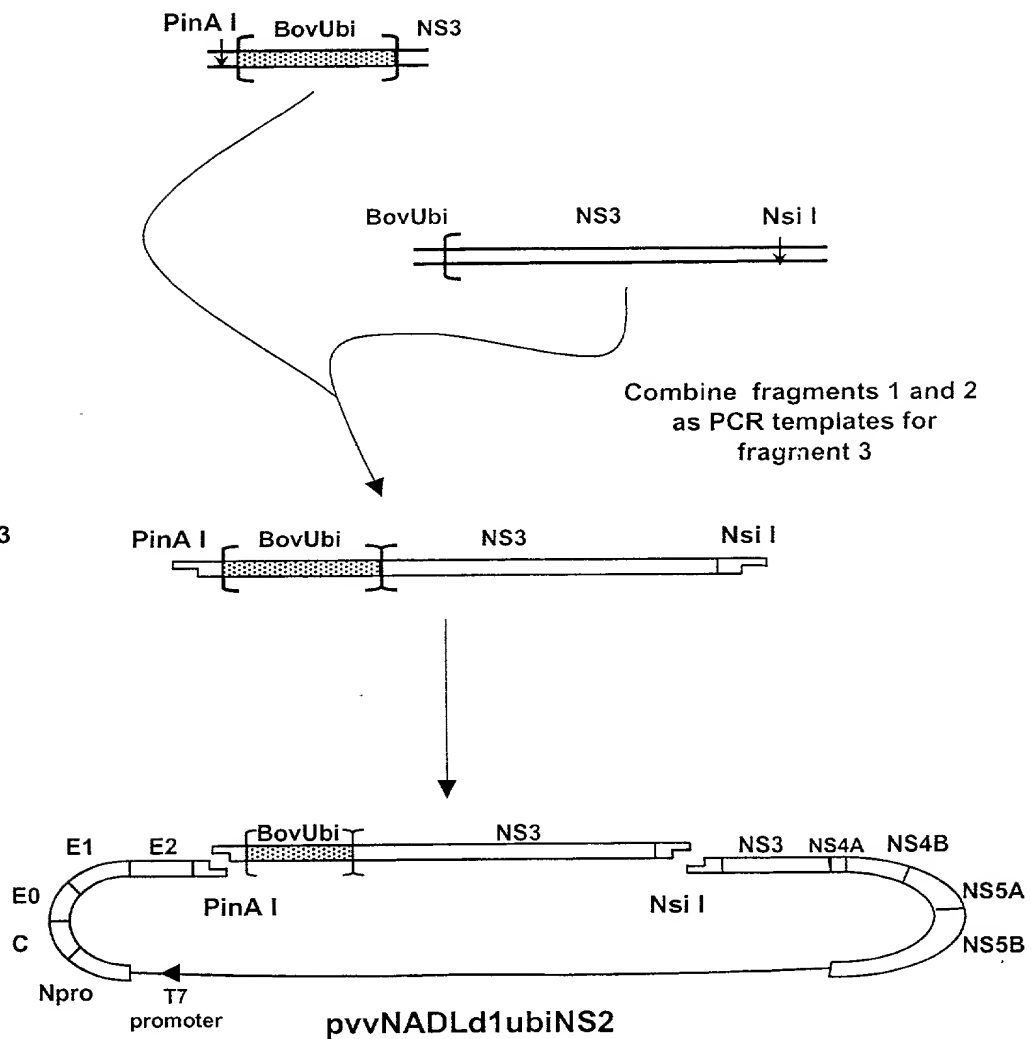
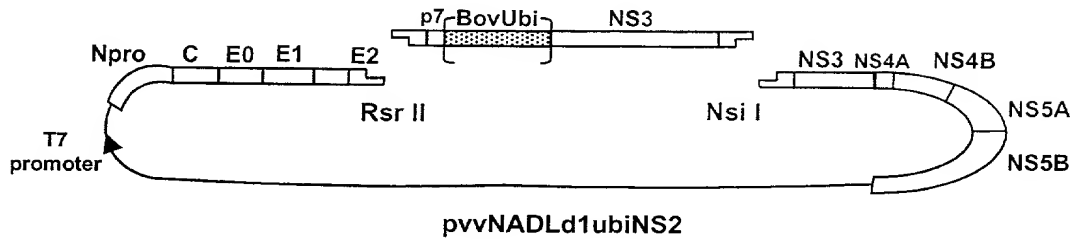


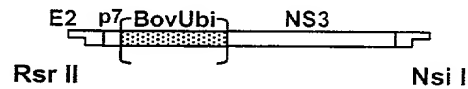
Figure 1A

B. Construction of p15aD1ubiNS2

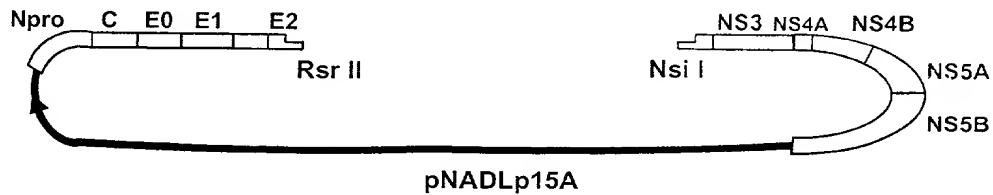
1. Restrict pvvNADLd1ubiNS2 with Rsr II and Nsi I



2. Isolate Rsr II and Nsi I fragment from pvvNADLd1ubiNS2



3. Ligate fragment with pNADLp15A restricted with Rsr II and Nsi I



4. Plasmid p15aD1ubiNS2

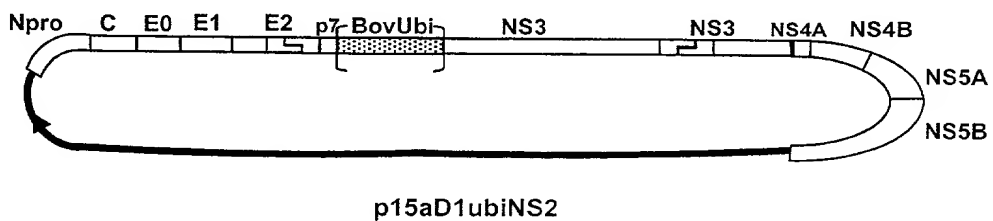
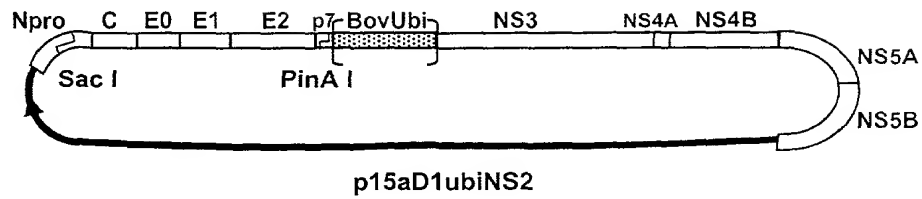


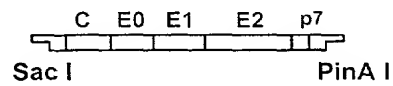
Figure 1B

C. Construction of p15aDI

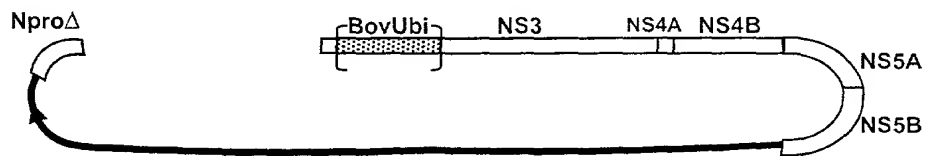
1. Restrict plasmid p15aD1ubiNS2 with Sac I and PinA I



Discard insert



2. Create blunt ends



3. Religate to create p15aDI

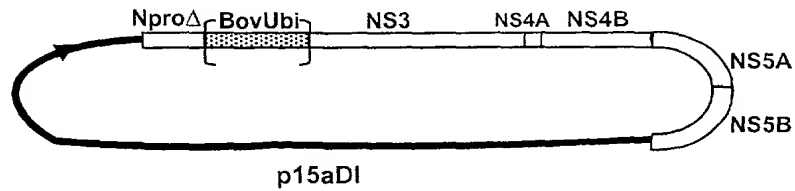


Figure 1C

D. Construction of pBVDdN6

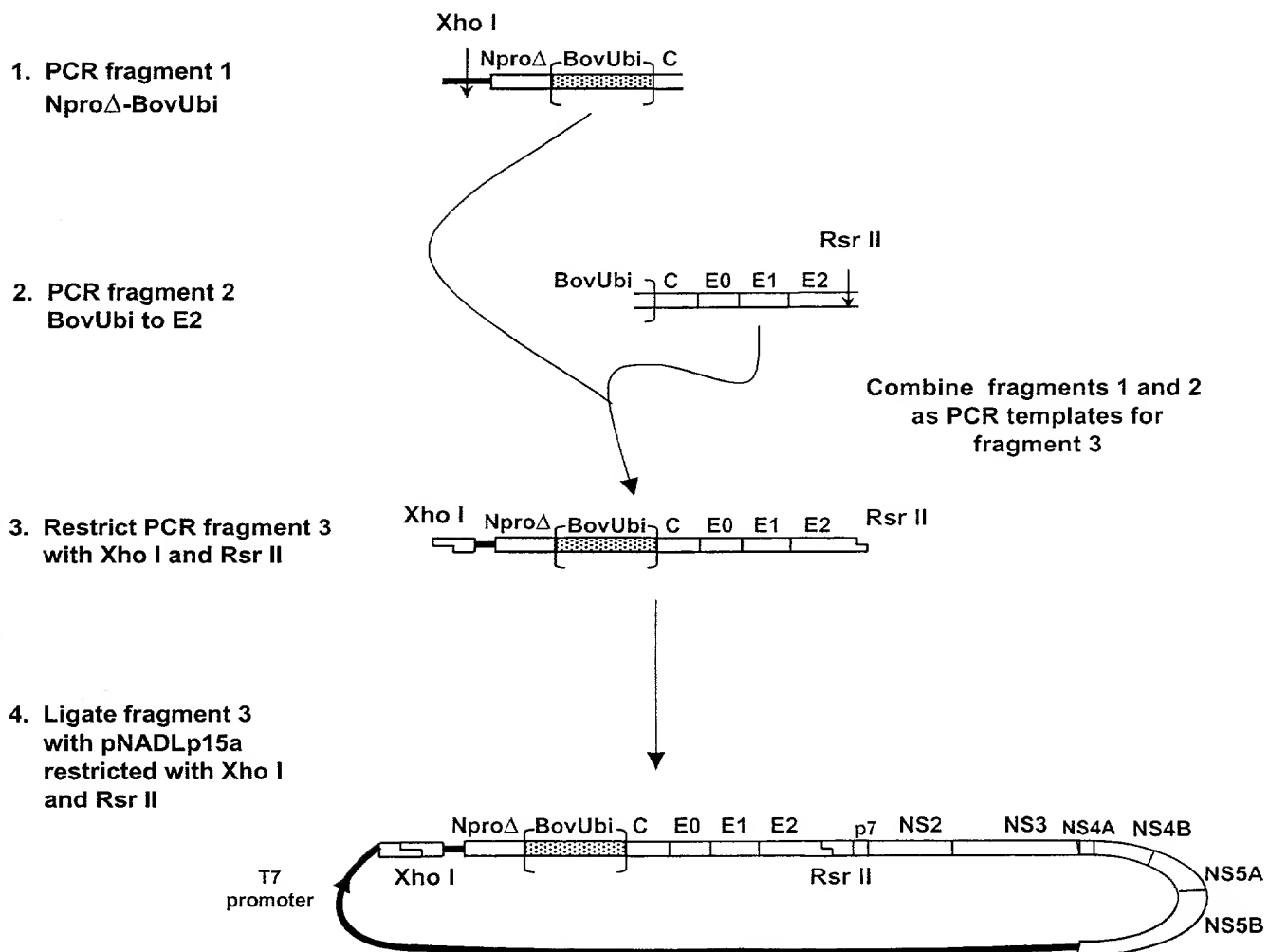


Figure 1D

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SEQ ID NO:11

FIGURE 2A

SHEET 3 OF 3

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FIGURE 2B

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FIGURE 2B

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FIGURE 2B

SHEET 4 OF 4

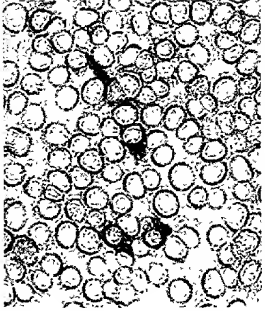
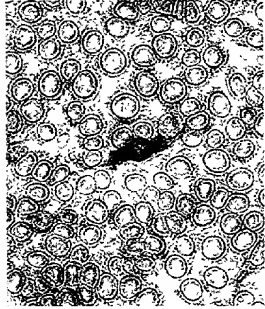
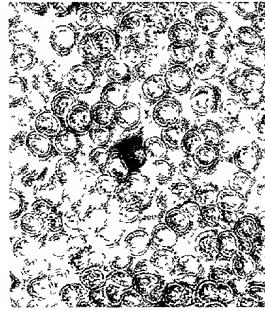
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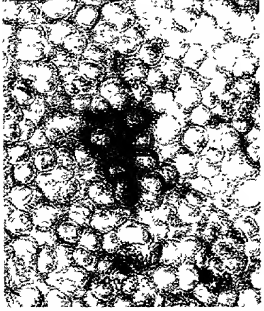
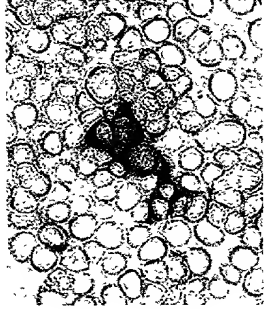
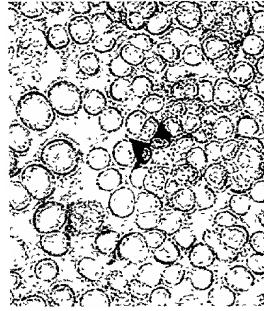
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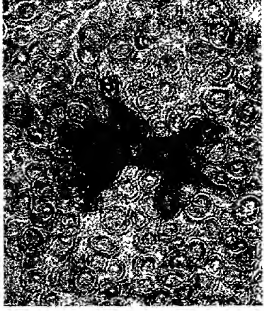
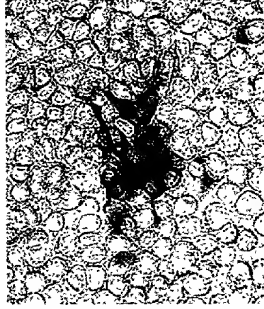
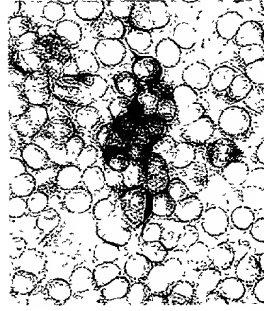
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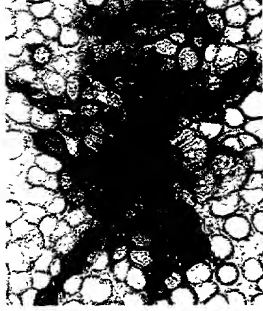
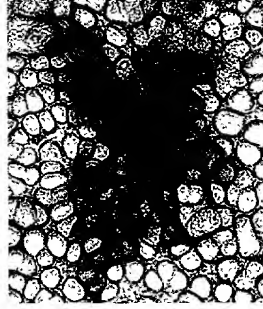
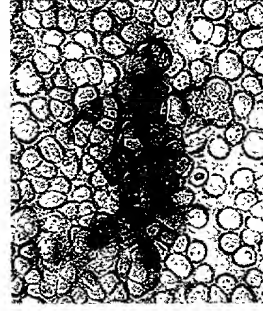
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24hrs



32hrs



48hrs

FIGURE 3

Growth curve of BVDV mutants in MDBK cell system

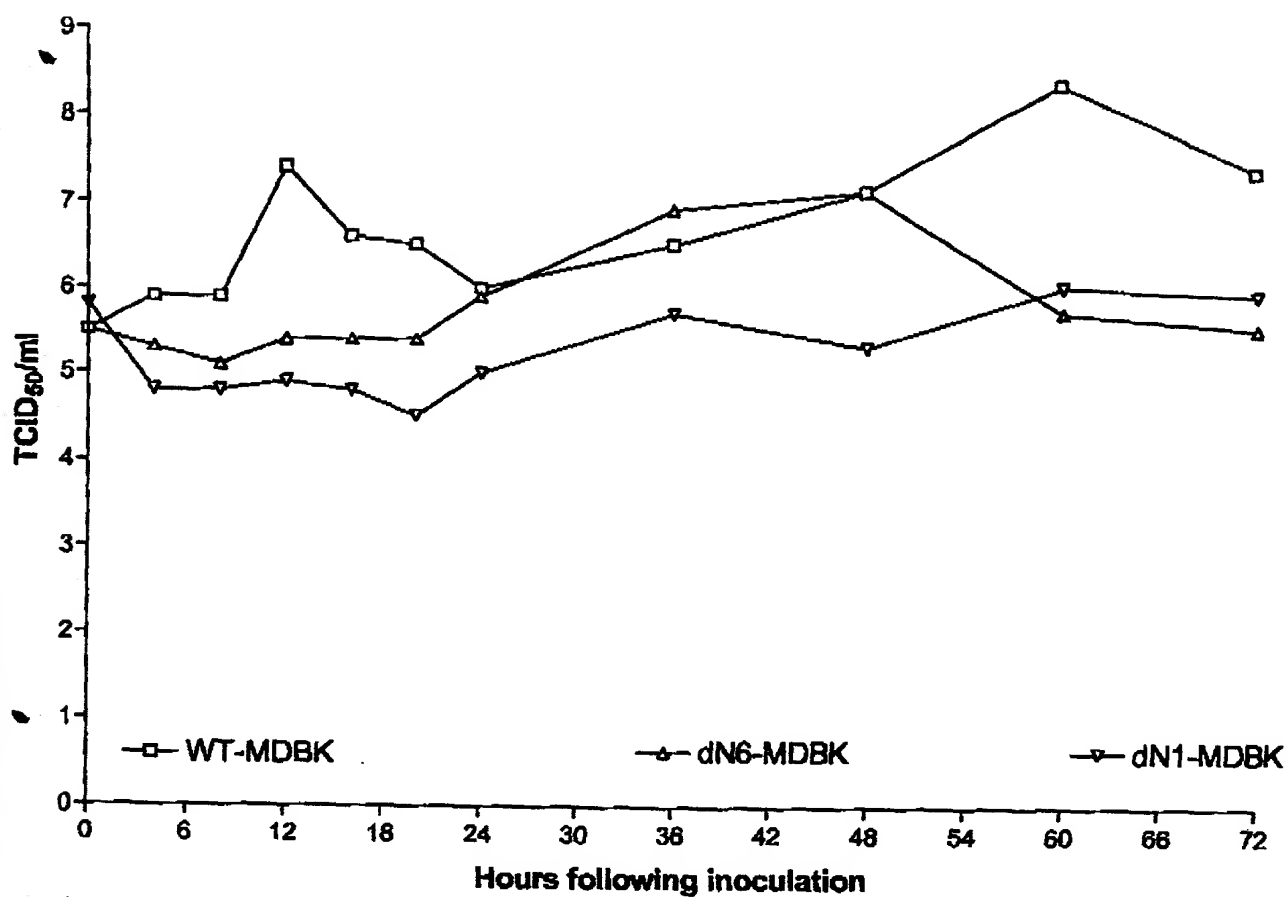


FIGURE 4